ORIGINAL

Simultaneous detection of *Bacteroides fragilis* group species by *leuB*-directed PCR

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Abstract : *Bacteroides* species, saccharolytic Gram-negative obligate anaerobes, are frequently isolated from human infections such as peritonitis, abscesses and bacteremia. Among the species in the genus *Bacteroides*, the species called "*B. fragilis* group" are particularly involved in human infections and are medically important because they account for a major part of anaerobic isolates from clinical specimens. The purpose of this study was to develop PCR primers that specifically and simultaneously amplify the β-isopropylmalate dehydrogenase gene *leuB* in *B. fragilis* group species. We determined partial nucleotide sequences of *leuB* genes and compared them in seventeen strains of nine *B. fragilis* group species, and the regions that are conserved among *Bacteroides* strains but different from other species were used as a *B. fragilis* group-specific PCR primer set, BacLBF-BacLBR. Specificity tests of the primer set using 52 phenotypically characterized strains and 75 isolates from rat feces showed only one case each of false-positive and false-negative. The detection limit of the *leuB*-directed PCR using BacLBF and BacLBR was 3.9×10^3 colony-forming units. These results indicate that *leuB* amplification using BacLBF and BacLBR is a useful tool for rapid diagnosis of *Bacteriodes* infection and for rapid differential diagnosis of anaerobic infections. J. Med. Invest. 52 : 101-108, February, 2005

Keywords **:** *Bacteroides, leuB, PCR, rapid detection*

INTRODUCTION

Bacteroides are saccharolytic Gram-negative obligate anaerobes that are most predominant in the human colon (1). The representative species of the genus *Bacteroides* is *B. fragilis*, and ten species that are phylogenetically close to *B. fragilis* have been defined as" *B. fragilis* group "(2). The *B. fragilis* group species are frequently isolated from mixed aerobic-anaerobic infections such as intra-abdominal, diabetic foot and soft tissue infections, though their virulence potentials are different. *B. fragilis* is regarded as the most virulent species among *Bacteroides* species and often causes severe bacteremia (3, 4). Although the growth rate of *Bacteroides* species is faster than that of other obligate anaerobes, identification of *Bacteroides* species by the traditional anaerobic culture method based on carbohydrate fermentation patterns is laborious, and use of this procedure sometimes results in misidentification. These conditions often delay the useful application of species identification and susceptibility data for patients. Therefore, PCR-based rapid and accurate identification methods in which specific genes such as glutamine synthetase gene (5) and neuraminidase gene (6, 7) are amplified have been developed for *B. fragilis*. Results obtained by using these methods showed that these methods have sufficient levels of specificity and sensitivity to be used for clinical speci-

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mens but that they provide limited information when species other than *B. fragilis* are contained in the specimens. A recent study on isolation rates of *B. fragilis* group species and antimicrobial susceptibility patterns demonstrated that the isolation rate has changed in the past ten years and that the increase in non-*B. fragilis* species has influenced the overall antimicrobial susceptibility of *Bacteroides* isolates (8). It is therefore important to develop a rapid and accurate method for identifying non-*B. fragilis* species for empirical antimicrobial therapy used by clinicians for treatment of mixed aerobic-anaerobic infections.

The aim of this study was to develop a PCR-based method for the simultaneous detection of all *B. fragilis* group species. For this purpose, we chose β-isopropylmalate dehydrogenase gene (*leuB*), which is involved in leucine biosynthesis, as a target because *B. fragilis* group species are leucine prototrophs and thought to have a complete set of genes for the leucine biosynthetic pathway (9).

MATERIALS AND METHODS

Bacterial strains

Bacterial strains used in this study are as follows. The anaerobes used were 9 strains of *B. fragilis* (2 laboratory strains, ATCC25285 and ATCC43859, and 7 clinical isolates), 6 strains each of *B. distasonis* (ATCC8503 and 5 clinical isolates) and *B. vulgatus* (ATCC8482 and 5 clinical isolates), 5 strains of *B. thetaiotaomicron* (ATCC 29148 and 4 clinical isolates), 4 strains of *B. uniformis* (2 laboratory strains, ATCC8492 and BU1001, and 2 clinical isolates), 3 strains of *B. ovatus* (ATCC8483 and 2 clinical isolates), and one strain each of *B. caccae* (JCM 9498), *B. eggerthii* (ATCC27754), *B. merdae* (JCM9497), *B. stercoris* (JCM9496), *Porphyromonas gingivalis* (381), *Por. asaccharolytica* (NCTC9337), *Por. endodontalis* (ATCC 35406), *Prevotella corporis* (JCM8480), *Prevotella melaninogenica* (ATCC25845), *Clostridium perfringens* (GAI 0668), *Eubacterium limosum* (ATCC8480), and *Fusobacterium nucleatum* (F-1). The aerobes used in this study were one strain each of *Bacillus subtilis* (T), *Staphylococcus aureus* (209P), *Staphylococcus epidermidis* (IID 866), *Streptococcus pyogenes* (UTB111), *Klebsiella pneumoniae* (KUB13), *Enterobacter aerogenes* (UTB7), *Proteus vulgaris* (KUB22), *Serratia marcescens* (UTB66), *Pseudomonas aeruginosa* (UTC55), and *Escherichia coli* (W3110). All anaerobic bacteria were cultured on GAM agar plates (Nissui Pharmaceutical Co., Tokyo, Japan) under anaerobic conditions. Aerobes except for *Streptococcus pyogenes* were cultured on Luria-Bertani (LB) agar plates (10). *Streptococcus pyogenes* was cultured on a 5% sheep blood agar plate.

Preparation of bacterial cells for PCR

Several colonies of each strain grown on GAM, LB, or 5% sheep blood agar plates were suspended in 1.0 ml of TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) and heated at 100°C for 10 min. After centrifugation at 12,000 *g* for 1 min, 10 μl of the supernatant was collected and used as a template for the PCR amplification of *leuB*. The mid-logarithmic phase culture (1.0 ml) of *B. fragilis* strain ATCC25285 in GAM broth was centrifuged, washed in 1.0 ml of TE buffer, and resuspended in 1.0 ml of TE. Serial 10-fold dilutions were prepared to give appropriate colony forming units (CFU), and they were lysed by heating at 100°C for 10 min. The supernatant (10 μl) of each dilution after centrifugation at 12,000 *g* for 1 min was used as a template to determine the sensitivity of *leuB*-PCR

PCR amplification and partial sequencing of leuB *genes from* B. fragilis *group species*

Oligonucleotide primers used in this study are listed in Table 1. Three kinds of primer sets (LBF22-LBR 44, LBF22-LBR55, and BacLBF-BacLBR) were used for amplification of *leuB*. PCR amplification of *leuB* was performed using 10 μl of heated cell supernatant of each strain, 0.5 μM of each oligonucleotide primer, and 2.5 units of *AmpliTaq* Gold (Perkin-Elmer Co., CT, U.S.A.) in the following setting: pre-heating (96°C for 10 min), 40 cycles of DNA denaturation (96°C for

Table 1. Oligonucleotide primers used for PCR amplification of *leuB*.

Primer	Sequence (5' to 3')*	Location **
LBF22	TGYGARAARTTYGGICA	85-101
LBR44	CCYTCIACYTGIATYTC	982-998
LBR55	ATRTARTCIACIATCCA	1033-1049
BacLBF	TGGCIATGCGYAARAA	281-296
BacLBR	CCCATRGARCCRCTGAT	754-770

* Y=T or C, R=A or G, and I=inosine.

** The location in the nucleotide sequence of *B. fragilis* strain YCH 46 *leuB* gene (13) is shown.

1 min)/primer annealing (50°C for 1 min)/DNA extension (72°C for 1 min). PCR products were separated by 2.0% agarose-gel electrophoresis and visualized by ethidium bromide staining. The LBF 22-LBR 55-generated *leuB* products were excised from agarose gel and purified using a QIA quick gel extraction kit (QIAGEN, Inc., CA, U.S.A.) according to the manufacturer's instructions. DNA concentration was determined spectrophotometrically, and 200 ng of each purified product was used for sequencing analysis. Nucleotide sequences of the internal portions of *leuB* genes of *B. fragilis* group species were determined on both strands with an automated sequencer (Genetic Analyzer 310, Applied Biosystems Inc., Foster City, Calif., U.S.A.) using BigDye terminator chemistry (Applied Biosystems). Nucleotide sequences obtained were aligned by using the multiple sequence alignment program Clustal W (11). The *B. fragilis* group-specific regions within *leuB* were determined by homology searches of the sequences obtained against public databases by BLASTN (12).

Isolation of bacteria from rat feces

One piece of fresh rat feces (*ca.* 0.1 g) was suspended in anaerobic solution (33 mM KH_2PO_4, 42 mM Na_2HPO_4, 0.05% L-cystein-HCl, 0.05% Tween 80, and 0.1% agar), and serial 10-fold dilutions were prepared with the anaerobic solution. A part of each dilution (0.1ml) was plated on GAM agar plates and incubated anaerobically for 48 hrs. Seventy-five colonies were randomly selected and isolated on fresh GAM agar plates. Gram-staining was performed on all of the isolates, and biochemical tests using an API 20 A system (Bio Mérieux S.A., France) were performed on obligate anaerobes for species identification.

Nucleotide sequence accession numbers

The partial *leuB* sequences that we determined in this study have been assigned the following GenBank accession numbers : AB196153 (*B. caccae* strain JCM 9498), AB196154 (*B. distasonis* strain ATCC8503), AB196155 (*B. eggerthii* strain ATCC27754), AB196149 (*B. fragilis* strain YCH1), AB196148 (*B. fragilis* strain ATCC25285), AB196150 (*B. fragilis* strain YCH46), AB196152 (*B. ovatus* strain ATCC8483), AB196151 (*B. ovatus* strain KYU7), AB196156 (*B. stercoris* strain JCM9496), AB196157 (*B. thetaiotaomicron* strain ATCC29148), AB196158 (*B. thetaiotaomicron* strain KYU12), AB196159 (*B. thetaiotaomicron* KYU16), AB196160 (*B. uniformis* strain ATCC8492), AB196164 (*B. uniformis* strain BU1001), AB196163 (*B. uniformis* strain OMC37), AB196161(*B. vulgatus* strain ATCC 8482), and AB196162 (*B. vulgatus* strain KYU10).

RESULTS

Partial sequencing of the **leuB** *genes of* **B. fragilis** *group species*

Among *Bacteroides* species, the nucleotide sequences

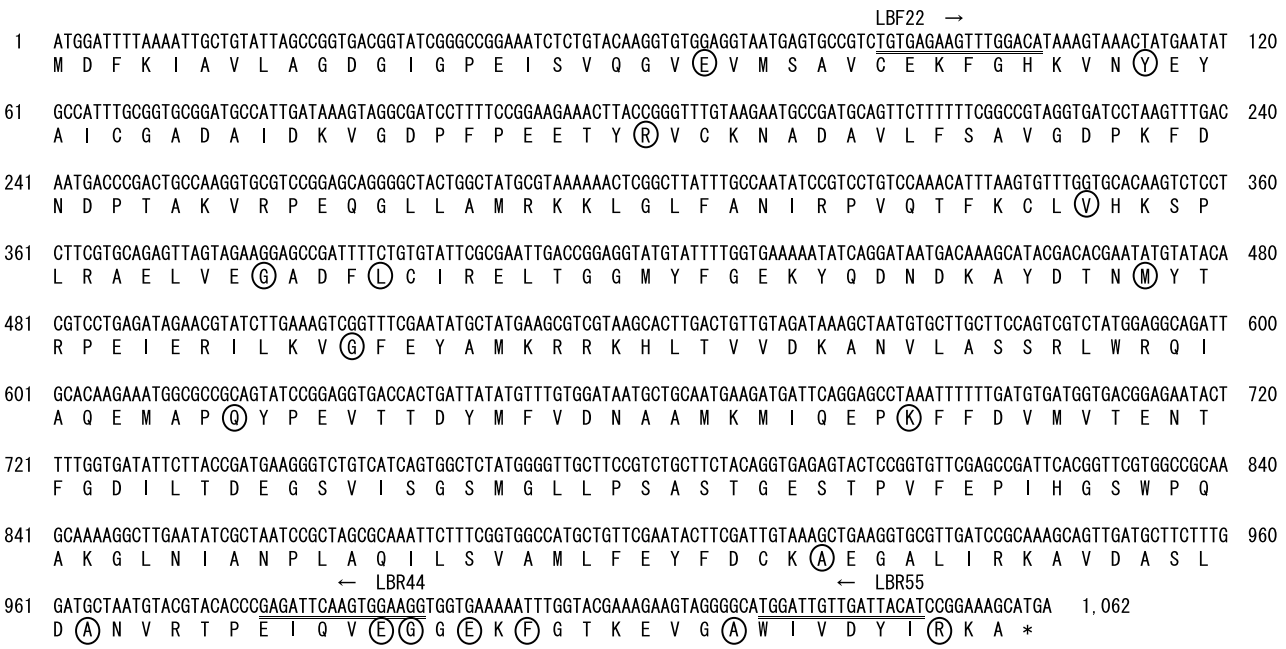

Fig. 1. The nucleotide sequence and the deduced amino acid sequence of the *leuB* gene from *B. fragilis* strain YCH46(13). The variable amino acids when compared with *B. thetaiotaomicron* strain VPI-5482 are circled. The annealing sites of the PCR primers (LBF22, LBR44, and LBR55) used for amplification of *leuB* from *B. fragilis* group species are double-underlined.

of *leuB* have been determined in *B. fragilis* strain YCH 46(13, 14) and *B. thetaiotaomicron* strain VPI-5482 (15). The nucleotide sequences of *leuB* in other *B. fragilis* group species must be obtained to design universal primers for all species in the *B. fragilis* group. Firstly, we designed PCR primers to amplify the *leuB* gene from *B. fragilis* group species other than *B. fragilis* and *B. thetaiotaomicron*. The predicted amino acid sequences of *leuB* in *B. fragilis* and *B. thetaiotaomicron* were highly conserved and showed 94.9% identity. The nucleotide sequence identity was 79.9% and a total of 213 mismatches were found within a 1,062-bp *leuB* sequence. Of those, 172 mismatches (80.8%) were present in the third position of the codon, and only 15 of them were responsible for amino acid substitutions. Therefore, we designed degenerate primers (LBF22, LBR44, and LBR55) from the conserved regions between *B. fragilis* and *B. thetaiotaomicron*, in which amino acids with low degeneracy are abundant (Fig. 1).

The results of amplification of *leuB* using the primer set LBF22-LBR44 or LBF22-LBR55 from type strains of 10 *B. fragilis* group species are shown in Table 2. The primer set LBF22-LBR55 generated the band of expected size (965 bp) from all of the type strains tested except for *B. merdae* strain JCM9497. The other primer set, LBF22-LBR44, also failed to yield a product from *B. merdae* strain JCM9497 despite the fact that both primer sets were able to generate an expected product from *B. distasonis*, which is a species close phylogenetically to *B. merdae*. We then amplified *leuB* genes from seventeen strains of nine species in the *B. fragilis* group except for *B. merdae* using the primer set LBF 22-LBR55 and determined the nucleotide sequences of the products. Overall nucleotide sequence identities among the strains tested ranged from 77 to 100%.

Design of PCR primers specific for species of the B. fragilis *group*

We aligned the partial *leuB* sequences from seventeen strains obtained as described above and searched for the conserved region among the species to design specific and universal primers for *B. fragilis* group species. Degenerate PCR primers, BacLBF and BacLBR, were synthesized on the basis of nucleotide sequences at positions 281-296 and 754-770, respectively, to be used for specific and simultaneous PCR detection of *B. fragilis* group species (Table 1 and Fig. 2).

Specificity test of the PCR primer set

To evaluate the specificity of the PCR primer set, BacLBF-BacLBR, we amplified *leuB* genes from 52 strains (37strains of the *B. fragilis* group, 5 strains of species closely related to the *B. fragilis* group, and 10 strains of unrelated aerobes) that had been stocked in our laboratory. As shown in Fig. 3 and Table 3, the PCR primer set, BacLBF-BacLBR, produced a 490-bp band from all strains of the *B. fragilis* group species tested except for one strain of *B. distasonis*. In contrast, none of the other species generated a 490-bp band.

The specificity of BacLBF-BacLBR was further examined in a blind fashion using 75 isolates from rat feces (43 aerobes and 32 anaerobes) (Table 4). Although only two *B. fragilis* group species (*B. caccae* and *B. vulgatus*) were identified within the isolates, a *leuB* amplification product was obtained from all strains of the two species. On the other hand, none of the other isolates generated any product except for one Gram-positive rod. This fastidious Gram-positive isolate was lost during cultivation and could not be identified at a species level. These results indicated that the *leuB*-derected PCR using the primer set, BacLBF-BacLBR, is a useful diagnostic tool for rapid detection of medically important *B. fragilis* group species.

Sensitivity of the PCR

As shown in Fig. 4, we prepared serial 10-fold dilutions of the *B. fragilis* strain ATCC25285, which contained cells from 3.9×10^{1} to 3.9×10^{6} CFU and were used as templates for amplification of *leuB* with the primer set BacLBF-BacLBR. The detection limit of *leuB*-directed PCR using BacLBF and BacLBR was 3.9×10^{3} CFU.

DISCUSSION

B. fragilis group species are medically important Gram-negative obligate anaerobes and are often associated with a variety of human infections. Since local *Bacteroides* infection often causes severe bacteremia in immunocompromised patients and since *B. fragilis* group species are the most resistant to antimicrobial agents among all anaerobes, rapid and accurate differentiation of anaerobic isolates is needed for effective management of infected patients. However, phenotypic tests for anaerobes by traditional culture methods used in clinical laboratories are time-consuming. In addition, comparisons of the results from 16S rDNA sequence based-identification and phenotypic tests revealed that only 45.5% of *B. cacacae*, 53.3% of *B. ovatus*, 66.7% of *B. merdae*, and 78.6% of *B. thetaiotaomicron* were correctly identified by phenotypic tests (16). PCR-based rapid diagnosis has been targeted to *B. fragilis* because this species is the most virulent among *Bac-*

Table 2. Results of PCR amplification of *leuB* from *Bacteroides fragilis* group species.

Bacterial strain	Primer set used	
	LBF22-LBR44	LBF22-LBR55
B. caccae **JCM9498**	NT	+
B. distasonis **ATCC8503**	+	+
B. eggerthii **ATCC27754**	NT	+
B. fragilis **ATCC25285**	NT	+
B. merdae **JCM9497**	—	—
B. ovatus **ATCC8483**	NT	+
B. stercoris **JCM9496**	NT	+
B. thetaiotaomicron **ATCC29148**	NT	+
B. uniformis **ATCC8492**	NT	+
B. vulgatus **ATCC8482**	NT	+

NT, not tested.
Plus and minus indicate whether visible and invisible bands produced by the PCR, respectively.

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B. ovatus ATCC8483                  272  AAGGCTTGCTGGCTATGCGTAAGAAACTGGGTCTTTTCGCTAATATCCGT  321
B. ovatus KYU7                      272  AAGGTTTGTTGGCGATGCGTAAGAAATTGGGACTTTTCGCTAATATCCGT  321
B. caccae JCM9498                   272  AGGGATTACTGGCAATGCGTAAGAAGTTGGGACTTTTCGCTAACATCCGT  321
B. thetaiotaomicron ATCC29148       272  AGGGATTGCTGGCAATGCGTAAGAAATTGGGATTGTTTGCTAATATTCGT  321
B. thetaiotaomicron KYU12           272  AGGGATTGCTGGCAATGCGTAAGAAATTGGGATTGTTTGCTAATATTCGT  321
B. thetaiotaomicron KYU16           272  AGGGATTGCTGGCAATGCGTAAGAAATTGGGATTGTTTGCTAATATTCGT  321
B. vulgatus ATCC8482               272  AAGGTTTGCTGGCTATGCGTAAAAAATTAGGTTTGTTTGCCAATATCCGT  321
B. vulgatus KYU10                   272  AAGGTTTGCTGGCTATGCGTAAAAAATTAGGTTTGTTTGCCAATATCCGT  321
B. fragilis YCH1                    272  AGGGGCTACTGGCTATGCGTAAAAAACTCGGCTTATTTGCCAATATCCGT  321
B. fragilis YCH46                   272  AGGGGCTACTGGCTATGCGTAAAAAACTCGGCTTATTTGCCAATATCCGT  321
B. fragilis ATCC25285               272  AGGGGCTACTGGCTATGCGTAAAAAACTCGGCTTATTTGCCAATATCCGT  321
B. eggerthii ATCC27754              272  AGGGCTTGCTGGCTATGCGTAAAAAACTGGGGCTGTTTGCCAACATCCGC  321
B. stercoris ATCC43183             272  AAGGACTGCTGGCTATGCGCAAGAAGTTGGGGCTGTTTGCCAATATCCGT  321
B. uniformis ATCC8492              272  AGGGCTTGCTGGCTATGCGCAAGAAACTGGGACTGTTTGCCAACATCCGT  321
B. uniformis BU1001                 272  AGGGCTTGCTGGCTATGCGCAAGAAACTGGGACTGTTTGCCAACACTTGT  321
B. uniformis OMC37                  272  AGGGCTTGCTGGCTATGCGCAAGAAACTGGGACTGTTTGCCAACATCCGT  321
B. distasonis ATCC8503              272  AAGGTCTTTTGGCGATGCGTAAGAAGTTGGGCCTGTTCGCTAATATCCGT    1
                                         * **  *  **** ***** ** **  * **  * ** ** ** *  *
                                           BacLBF:TGGC|ATGCGYAARAA

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B. ovatus ATCC8483                  722  TCGGTGATATTCTGACTGACGAGGGTTCTGTAATCAGTGGTTCTATGGGC  771
B. ovatus KYU7                      722  TCGGTGATATTCTGACTGACGAAGGTTCTGTAATCAGTGGTTCTATGGGC  771
B. caccae JCM9498                   722  TCGGTGATATTCTGACCGATGAAGGTTCTGTAATCAGTGGTTCTATGGGA  771
B. thetaiotaomicron ATCC29148       722  TCGGGGATATTCTGACTGACGAAGGTTCTGTTATCAGCGGCTCTATGGGA  771
B. thetaiotaomicron KYU12           722  TCGGGGATATTCTGACTGACGAAGGTTCTGTTATCAGCGGCTCTATGGGA  771
B. thetaiotaomicron KYU16           722  TCGGGGATATTCTGACTGACGAAGGTTCTGTTATCAGCGGCTCTATGGGA  771
B. vulgatus ATCC8482               722  TTGGTGATATTCTGACAGATGAAGGCTCTGTTATCAGTGGCTCTATGGGA  771
B. vulgatus KYU10                   722  TTGGTGATATTCTGACAGATGAAGGCTCTGTTATCAGTGGCTCTATGGGA  771
B. fragilis YCH1                    722  TTGGTGATATTCTTACCGATGAAGGGTCTGTCATCAGTGGCTCTATGGGG  771
B. fragilis YCH46                   722  TTGGTGATATTCTTACCGATGAAGGGTCTGTCATCAGTGGCTCTATGGGG  771
B. fragilis ATCC25285               722  TTGGTGATATTCTTACCGATGAAGGGTCTGTCATCAGTGGCTCTATGGGG  771
B. eggerthii ATCC27754              722  TCGGAGATATTCTGACCGATGAAGGCTCCTGCATCAGCGGTTCTATGGGA  771
B. stercoris ATCC43183             722  TCGGCGATATTCTGACCGATGAAGGCTCCTGCATCAGCGGCTCCATGGGC  771
B. uniformis ATCC8492              722  TTGGTGATATTTTGACGGACGAGGGTTCCTGCATCAGTGGCTCTATGGGA  771
B. uniformis BU1001                 722  TTGGTGATATTTTGACGGACGAGGGTTCCGTCATCAGTGGCTCTATGGGA  771
B. uniformis OMC37                  722  TTGGTGATATTTTGACGGACGAGGGTTCCTGCATCAGTGGCTCTATGGGA  771
B. distasonis ATCC8503                2  TTGGTGATATCTTGACGGATGAGGGTTCTTGCATCAGCGGCTCTATGGGC  771
                                         * ** *****  * ** ** ** ** **    ***** ** ** *****
                                                                BacLBR:TAGTCRCCRAGRTACCC
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Fig. 2. Alignment of partial *leuB* sequences of *B. fragilis* group species. Numbers on both sides indicate the nucleotide positions in *leuB* of *B. fragilis* strain YCH46. The annealing sites of the primers BacLBF and BacLBR are indicated and double-underlined. Asterisks indicate the conserved nucleotides among all of the strains tested.

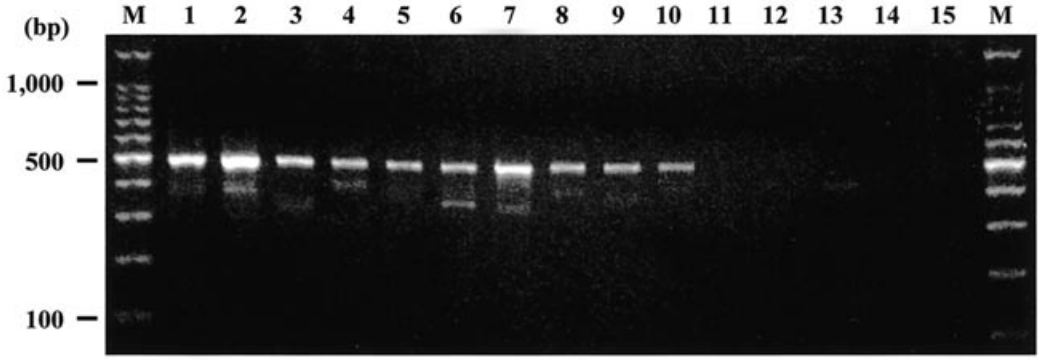

Fig. 3. Specificity of the *leuB*-directed PCR using BacLBF and BacLBR. Lanes : 1, *B. caccae* JCM9498 ; 2, *B. distasonis* ATCC8503 ; 3, *B. eggerthii* ATCC27754 ; 4, *B. fragilis* ATCC25285 ; 5, *B. merdae* JCM9497 ; 6, *B. ovatus* ATCC8483 ; 7, *B. stercoris* JCM9496 ; 8, *B. thetaiotaomicron* ATCC29148 ; 9, *B. uniformis* ATCC8492 ; 10, *B. vulgatus* ATCC8482 ; 11, *Por. asaccharolytica* ATCC25260 ; 12, *Por. gingivalis* 381 ; 13, *Prevotella corporis* JCM8529 ;14, *Prevotella melaninogenica* ATCC25845 ; 15, *Escherichia coli* W3110 ; M, molecular size markers.

Table 3. Specificity tests of the oligonucleotide primers, BacLBF and BacLBR, using phenotypically characterized bacterial strains.

Species classification	No. of strains	*leuB*-directed PCR	
		No. of positive	No. of negative
***Bacteroides fragilis* group**			
B. caccae	1	1	0
B. distasonis	6	5	1
B. eggerthii	1	1	0
B. fragilis	9	9	0
B. merdae	1	1	0
B. ovatus	3	3	0
B. stercoris	1	1	0
B. thetaiotaomicron	5	5	0
B. uniformis	4	4	0
B. vulgatus	6	6	0
Species closely related to *B. fragilis* group			
Porphyromonas asaccharolytica	1	0	1
Por. gingivalis	1	0	1
Por. endodontalis	1	0	1
Prevotella corporis	1	0	1
Prevotella melaninogenica	1	0	1
Unrelated aerobic species	10	0	10

teroides and accounts for 63% of all the *B. fragilis* group isolates (17). However, a recent survey has revealed that isolation rates of *Bacteroides* species other than *B. fragilis* have been increasing in the past ten years. It is therefore important to develop a rapid and accurate technique for identification of all *B. fragilis* group species for appropriate treatment of anaerobic infections.

Liu *et al*. have recently reported *B. fragilis* group-specific PCR primers designed on the basis of the 16S rDNA sequence (16). However, due to the high sequence similarities of 16S rDNA among closely related species, this primer set also reacts to several strains of *Porphyromonas* and *Prevotella*. In this study, we synthesized *B. fragilis* group-specific PCR primers (BacLBF-BacLBR) on the basis of β-isopropylmalate dehydrogenase gene. This primer set showed high specificity for *B. fragilis* group species and did not cross-react with closely related species in *Porphyromonas* and *Prevotella*.

The minimum level of *leuB*-directed PCR using BacLBF and BacLBR was 3.9×10^3 cells. It seems to be difficult to apply *leuB*-directed PCR for direct detection of *Bacteroides* in specimens that contain relatively small numbers of bacterial cells, such as in a blood sample from a patient with bacteremia. However, the PCR will be a useful diagnostic tool when it is applied to specimens that contain relatively large numbers of bacterial cells such as samples of pus or drainage fluids. It will also be

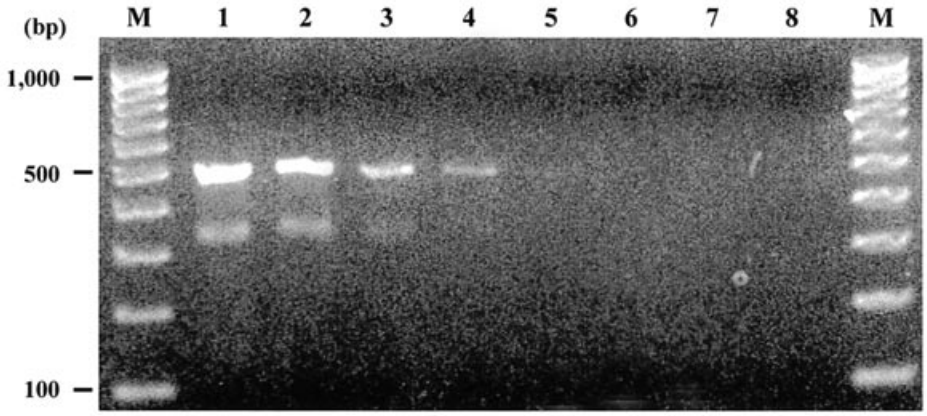

Fig. 4. Sensitivity of the *leuB*-directed PCR using BacLBF and BacLBR on the basis of viable cell number of *B. fragilis* strain ATCC 25285. Lanes: 1, 3.9×10^{6}; 2, 3.9×10^{5}; 3, 3.9×10^{4}; 4, 3.9×10^{3}; 5, 3.9×10^{2}; 6, 3.9×10^{1}; 7, 3.9×10^{0}; 8, 3.9×10^{-1}; M, molecular size markers.

Table 4. Specificity tests of the oligonucleotide primers, BacLBF and BacLBR, using fecal isolates from a rat.

Isolate classification *	No. of isolates tested	*leuB*-directed PCR	
		No. of positive	No. of negative
Aerobes			
Gram-positive cocci	8	0	8
Gram-positive rods	24	1	23
Gram-negative rods	11	0	11
Anaerobes			
Gram-positive rods			
Bifidobacterium spp	3	0	3
Lactobacillus fermentum	1	0	1
Unidentified	2	0	2
Gram-negative rods			
Bacteroides caccae	15	15	0
Bacteroides vulgatus	9	9	0
Porphyromonas gingivalis	2	0	2

*Anaerobic isolates were identified at the species level by biochemical tests using an API20 A system.

useful to confirm the results obtained by traditional biochemical tests. The diagnostic potentials of the PCR developed in this study are expected to be increased by simultaneous use with species-specific primer sets such as *B. fragilis*-specific primers designed from *glnA* (5) or *nanH* (6, 7).

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